Experiments with soils.

Two types of soil were used—a garden loam from Sydney University and a sub-Antarctic peat from Macquarie Island. The protozoa numbers were estimated by the technique suggested and by inoculating a series of soil dilutions into (1) lucerne broth (100 g. finely ground lucerne chaff boiled in water for 30 minutes, filtered, and filtrate made up to one litre with tap water, pH adjusted to 7·0–7·2) and (2) mannitol soil extract agar (Allen, 1949).

The results, calculated from McGrady's tables, are summarized in Table 3.

Except in the case where University loam was inoculated into mannitol soil extract agar, greater numbers of protozoa were detected with direct microscopy than with several commonly employed culture techniques. This was especially noticeable with the soil from Macquarie Island. The differences in numerical distribution of the various groups shown by the different techniques is particularly striking.

Discussion.

Most staining techniques used for counting soil microorganisms by direct microscopy have been based on the fact that acid dyes do not stain soil colloids. This is satisfactory if a lens of high magnification is used. When lenses of low magnification are desirable, it is better to have a strong contrast between the background and the microorganisms. This chromatic property has been used in the Ziehl-Neelsen stain for the detection of *Mycob. tuberculosis* in sputum, using the advantage of a strong contrast between blue and red. To obtain the desirable contrast in the present technique a green-red combination was utilized, which is more restful during prolonged observation than blue-red. The background colour is obtained with methyl green. *A priori*, the use of a basic dye is not indicated for staining soil suspensions for counting the microflora. Erythrosin stains microorganisms red and this dye will be retained sufficiently to produce a pink colour provided the basic dye treatment is not prolonged. Since the cytoplasm of protozoa has a strong affinity for acid dyes, it is stained intensively by erythrosin. Methyl green is a basic dye which has an affinity for cell nuclei and soil particles. The nucleus is stained by erythrosin and methyl green so that it appears pink.

The Gram stain has the added advantage of securing extra information on the soil microflora. The Gram technique described can also be applied to Jones and Mollison's agar film technique for counting soil microorganisms. Since there is no heating, it does not damage the agar film. The method has advantages over Jensen's (1934) technique, which is more difficult to use and may damage the agar film when the heat treatment is applied.

The slides used must be perfectly clean and handled with care. Human skin cells may be found very commonly on slides if one does not take the precaution of avoiding the surfaces of the slides with one's fingers. However, such cells are readily recognizable and do not result in false positives being recorded.

The use of grease pencil for delimiting an area on the slide has two advantages: 1. The suspension will not run on the slide; 2. The edge of the preparation is well defined thus facilitating microscopic examination.

The use of agar fluid for suspending the soil particles is important. It fixes the protozoa strongly on the slide and eliminates the loss of cells during washing. With plain water, 90% losses occurred in some cases. Agar, in low concentration, does not solidify at room temperature and is straightforward to use in the preparation of soil dilutions. The viscosity of the fluid is higher than water and makes soil suspensions more homogeneous, so that often a better distribution of the contained protozoa results.

The present technique has been compared with other microscopic methods. Fluorescence microscopy using orange acridin is of no use (Strugger, 1948), as soil particles fluoresce red and are not in contrast with the protozoa. Dark field illumination is not suitable because of the degree of light reflection caused by the particles of